Claims

1. (previously presented) A method for selecting a hybridization oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying a predetermined number of non-identical oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being chosen to sample a length of said nucleotide sequence, wherein said non-identical oligonucleotides are of identical length N and are spaced one nucleotide apart, said predetermined number comprising L-N+1 oligonucleotides, where L is the length of the hybridizable sequence,

(b) determining and evaluating for each of said oligonucleotides at least one parameter that is predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence,

(c) selecting a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides based on an examination of said parameter,

(d) identifying oligonucleotides in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and

(e) selecting, for a cluster, a hybridization oligonucleotide wherein the hybridization of said hybridization oligonucleotide is predicted by the presence of said hybridization oligonucleotide in said cluster.

2. (original) A method according to Claim 1 which comprises ranking said oligonucleotides of step (d) based on the size of said clusters of oligonucleotides.

Claims 3-4 (canceled).

5. (original) A method according to Claim 1 wherein said parameter is selected from the group consisting of composition factors, thermodynamic factors, chemosynthetic efficiencies and kinetic factors.

6. (original) A method according to Claim 1 wherein said parameter is a composition factor selected from the group consisting of mole fraction (G+C), percent (G+C), sequence complexity, and sequence information content.

7. (original) A method according to Claim 1 wherein said parameter is a thermodynamic factor selected from the group consisting of predicted duplex melting temperature, predicted enthalpy of duplex formation, predicted entropy of duplex formation, predicted free energy of duplex formation, predicted melting temperature of the most stable intramolecular structure of the oligonucleotide or its complement, predicted enthalpy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted entropy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted free energy of the most stable intramolecular structure of the oligonucleotide or its complement, predicted melting temperature of the most stable hairpin structure of the oligonucleotide or its complement, predicted enthalpy of the most stable hairpin structure of the oligonucleotide or its complement, predicted entropy of the most stable hairpin structure of the oligonucleotide or its complement, predicted free energy of the most stable hairpin structure of the oligonucleotide or its complement, thermodynamic partition function for intramolecular structure of the oligonucleotide or its complement.

8. (original) A method according to Claim 1 wherein said parameter is a chemosynthetic efficiency selected from the group consisting of coupling efficiencies and overall efficiency of the synthesis of a target nucleotide sequence or an oligonucleotide probe.

9. (original) A method according to Claim 1 wherein said parameter is a kinetic factor selected from the group consisting of steric factors calculated via molecular modeling, rate constants calculated via molecular dynamics simulations, rate constants calculated via semi-empirical kinetic modeling, associative rate constants, dissociative rate constants, enthalpies of activation, entropies of activation, and free energies of activation.

10. (previously presented) A method according to Claim 1 wherein said parameter is derived from a factor by mathematical transformation of said factor wherein said factor is predictive of the ability of an oligonucleotide to hybridize with a target nucleotide sequence.

11. (original) A method according to Claim 1 which comprises ranking said clustered oligonucleotides of step (d) based on the size of said clusters of oligonucleotides and selecting a subset of said clustered oligonucleotides.

12. (original) A method according to Claim 11 wherein said subset consists of any number of oligonucleotides within said cluster of oligonucleotides.

13. (original) A method according to Claim 11 wherein the subset of said clustered oligonucleotides are selected to statistically sample the cluster.

14. (original) A method according to Claim 13 wherein said statistical sample consists of oligonucleotides spaced at the first quartile, median and third quartile of the cluster of oligonucleotides.

15. (original) A method according to Claim 1 wherein said parameters are determined for said oligonucleotides by means of a computer program.

16. (original) A method according to Claim 1 wherein said oligonucleotides are attached to a surface.

17. (original) A method according to Claim 1 wherein said oligonucleotides are DNA.

18. (original) A method according to Claim 1 wherein said oligonucleotides are RNA.

19. (original) A method according to Claim 1 wherein said oligonucleotides contain chemically modified nucleotides.

20. (original) A method according to Claim 1 wherein said target nucleotide sequence is RNA.

21. (original) A method according to Claim 1 wherein said target nucleotide sequence is DNA.

22. (original) A method according to Claim 1 wherein said target nucleotide sequence contains chemically modified nucleotides.

23. (original) A method according to Claim 1 wherein said parameter is, for each oligonucleotide/target nucleotide sequence duplex, the difference between the predicted duplex

melting temperature corrected for salt concentration and the temperature of hybridization of each of said oligonucleotides with said target nucleotide sequence.

24. (previously presented) A method according to Claim 1 wherein step (c) comprises identifying a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides by establishing cut-off values for said parameter.

25. (previously presented) A method according to Claim 1 wherein said step (c) comprises identifying a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides by converting the values of said parameter into a dimensionless number by determining a dimensionless score for each parameter resulting in a distribution of scores having a mean value of zero and a standard deviation of one.

26. (Canceled)

27. (previously presented) A method according to Claim 25 which comprises optimizing a method according to calculation for said parameter based on said individual scores.

28. (previously presented) A method according to Claim 1 wherein step (b) comprises determining at least two parameters wherein the absolute value of a correlation coefficient between said parameters is less than 0.5.

29. (original) A method according to Claim 28 wherein said parameters are derived from a combination of factors by mathematical transformation of those factors.

30. (original) A method according to Claim 1 wherein step (b) comprises determining two parameters at least one of said parameters being the association free energy between a subsequence within each of said oligonucleotides and its complementary sequence on said target nucleotide sequence.

31. (original) A method according to Claim 30 wherein said subsequence is 3 to 9 nucleotides in length.

32. (original) A method according to Claim 30 wherein said subsequence is 5 to 7 nucleotides in length.

33. (original) A method according to Claim 30 wherein said subsequence is at least three nucleotides from the terminus of said oligonucleotides.

34. (original) A method according to Claim 30 wherein said subsequence is at least three nucleotides from a surface to which said oligonucleotides are attached.

35. (original) A method according to Claim 30 wherein said oligonucleotides are attached to a surface and said subsequence is at least five nucleotides from the terminus of said oligonucleotides that is attached to said surface and at least three nucleotides from the free end of said oligonucleotides.

36. (original) A method according to Claim 30 wherein the association free energy of the members of a set of subsequences within each of said oligonucleotides is determined and said subsequence having the minimum value is identified.

37. (original) A method according to Claim 1 which comprises including oligonucleotides that are adjacent to said oligonucleotides in said subset that are clustered along a region of said target nucleotide sequence.

38. (previously presented) A method according to Claim 1 which comprises (i) identifying a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides by establishing cut-off values for each of said parameters.

39. (original) A method according to Claim 1 which comprises determining the sizes of said clusters of step (d) by counting the number of contiguous oligonucleotides in said region of said hybridizable sequence.

40. (original) A method according to Claim 1 which comprises determining the sizes of said clusters of step (d) by counting the number of oligonucleotides in said subset that begin in a region of predetermined length in said hybridizable sequence.

Claims 41-101 (canceled).

102. (previously presented) A method for selecting a hybridization oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying a predetermined number of non-identical oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being chosen to sample a length of said nucleotide sequence,

(b) determining and evaluating for each of said oligonucleotides at least one parameter that is predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence,

(c) selecting a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides based on an examination of said parameter,

(d) identifying oligonucleotides in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and ranking said oligonucleotides based on the size of said clusters of oligonucleotides and

(e) selecting, for a cluster, a hybridization oligonucleotide wherein the hybridization of said hybridization oligonucleotide is predicted by the presence of said hybridization oligonucleotide in said cluster.

103. (previously presented) A method according to Claim 102 wherein said non-identical oligonucleotides are of identical length N.

104. (previously presented) A method according to Claim 103 wherein said non-identical oligonucleotides are spaced one nucleotide apart, said predetermined number comprising L-N+1 oligonucleotides, where L is the length of the hybridizable sequence.

105. (previously presented) A method according to Claim 102 wherein said parameter is selected from the group consisting of composition factors, thermodynamic factors, chemosynthetic efficiencies and kinetic factors.

106. (previously presented) A method according to Claim 102 which further comprises selecting a subset of said clustered oligonucleotides of step (d).

107. (previously presented) A method according to Claim 106 wherein said subset consists of any number of oligonucleotides within said cluster of oligonucleotides.

108. (previously presented) A method according to Claim 106 wherein the subset of said clustered oligonucleotides are selected to statistically sample the cluster.

109. (previously presented) A method according to Claim 102 wherein said parameters are determined for said oligonucleotides by means of a computer program.

110. (previously presented) A method according to Claim 102 wherein said oligonucleotides are attached to a surface.

111. (previously presented) A method according to Claim 102 wherein said oligonucleotides are DNA or RNA or comprise chemically modified nucleotides.

112. (previously presented) A method according to Claim 102 wherein said target nucleotide sequence is RNA or DNA or comprise chemically modified nucleotides.

113. (previously presented) A method according to Claim 102 wherein step (c) comprises identifying a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides by establishing cut-off values for said parameter.

114. (previously presented) A method according to Claim 102 wherein step (b) comprises determining two parameters at least one of said parameters being the association free energy between a subsequence within each of said oligonucleotides and its complementary sequence on said target nucleotide sequence.

115. (previously presented) A method according to Claim 114 wherein said subsequence is 3 to 9 nucleotides in length or 5 to 7 nucleotides in length.

116. (previously presented) A method according to Claim 114 wherein said subsequence is at least three nucleotides from the terminus of said oligonucleotides.

117. (previously presented) A method according to Claim 114 wherein said subsequence is at least three nucleotides from a surface to which said oligonucleotides are attached.

118. (previously presented) A method according to Claim 114 wherein said oligonucleotides are attached to a surface and said subsequence is at least five nucleotides from the terminus of said oligonucleotides that is attached to said surface and at least three nucleotides from the free end of said oligonucleotides.

119. (previously presented) A method according to Claim 102 which comprises including oligonucleotides that are adjacent to said oligonucleotides in said subset that are clustered along a region of said target nucleotide sequence.

120. (previously presented) A method according to Claim 102 which comprises determining the sizes of said clusters of step (d) by counting the number of contiguous oligonucleotides in said region of said hybridizable sequence.

121. (previously presented) A method according to Claim 102 which comprises determining the sizes of said clusters of step (d) by counting the number of oligonucleotides in said subset that begin in a region of predetermined length in said hybridizable sequence.

122. (previously presented) A method for selecting a hybridization oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying a predetermined number of non-identical oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being of identical length N and being chosen to sample a length of said nucleotide sequence ,

(b) determining and evaluating for each of said oligonucleotides at least one parameter that is predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence,

(c) selecting a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides based on an examination of said parameter,

(d) identifying oligonucleotides in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and

(e) selecting, for a cluster, a hybridization oligonucleotide wherein the hybridization of said hybridization oligonucleotide is predicted by the presence of said hybridization oligonucleotide in said cluster.

123. (previously presented) A method according to Claim 122 which comprises ranking said oligonucleotides of step (d) based on the size of said clusters of oligonucleotides.

124. (previously presented) A method according to Claim 122 wherein said non-identical oligonucleotides are spaced one nucleotide apart, said predetermined number comprising L-N+1 oligonucleotides, where L is the length of the hybridizable sequence.

125. (previously presented) A method according to Claim 122 wherein said parameter is selected from the group consisting of composition factors, thermodynamic factors, chemosynthetic efficiencies and kinetic factors.

126. (previously presented) A method according to Claim 122 which further comprises selecting a subset of said clustered oligonucleotides of step (d).

127. (previously presented) A method according to Claim 126 wherein said subset consists of any number of oligonucleotides within said cluster of oligonucleotides.

128. (previously presented) A method according to Claim 126 wherein the subset of said clustered oligonucleotides are selected to statistically sample the cluster.

129. (previously presented) A method according to Claim 122 wherein said parameters are determined for said oligonucleotides by means of a computer program.

130. (previously presented) A method according to Claim 122 wherein said oligonucleotides are attached to a surface.

131. (previously presented) A method according to Claim 122 wherein said oligonucleotides are DNA or RNA or comprise chemically modified nucleotides.

132. (previously presented) A method according to Claim 122 wherein said target nucleotide sequence is RNA or DNA or comprise chemically modified nucleotides.

133. (previously presented) A method according to Claim 122 wherein step (c) comprises identifying a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides by establishing cut-off values for said parameter.

134. (previously presented) A method according to Claim 122 wherein step (b) comprises determining two parameters at least one of said parameters being the association free energy between a subsequence within each of said oligonucleotides and its complementary sequence on said target nucleotide sequence.

135. (previously presented) A method according to Claim 134 wherein said subsequence is 3 to 9 nucleotides in length or 5 to 7 nucleotides in length.

136. (previously presented) A method according to Claim 134 wherein said subsequence is at least three nucleotides from the terminus of said oligonucleotides.

137. (previously presented) A method according to Claim 134 wherein said subsequence is at least three nucleotides from a surface to which said oligonucleotides are attached.

138. (previously presented) A method according to Claim 134 wherein said oligonucleotides are attached to a surface and said subsequence is at least five nucleotides from the terminus of said oligonucleotides that is attached to said surface and at least three nucleotides from the free end of said oligonucleotides.

139. (previously presented) A method according to Claim 122 which comprises including oligonucleotides that are adjacent to said oligonucleotides in said subset that are clustered along a region of said target nucleotide sequence.

140. (previously presented) A method according to Claim 122 which comprises determining the sizes of said clusters of step (d) by counting the number of contiguous oligonucleotides in said region of said hybridizable sequence.

141. (previously presented) A method according to Claim 122 which comprises determining the sizes of said clusters of step (d) by counting the number of oligonucleotides in said subset that begin in a region of predetermined length in said hybridizable sequence.

142. (previously presented) A method according to Claim 102 further comprising performing steps (a)-(e) under computer control.

143. (previously presented) A method according to claim 142 wherein the identified subset of oligonucleotide sequences is electronically transferred to an oligonucleotide array manufacturing system.

144. (previously presented) A method according to Claim 122 further comprising performing steps (a)-(e) under computer control.

145. (previously presented) A method according to claim 144 wherein the identified subset of oligonucleotide sequences is electronically transferred to an oligonucleotide array manufacturing system.

146. (previously presented) A computer system for conducting a method for selecting a hybridization oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) input means for introducing a target nucleotide sequence into said computer system,

(b) means for determining a number of non-identical oligonucleotides that are within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotide sequences being chosen to sample a length of said nucleotide sequence,

(c) memory means for storing said oligonucleotide sequences,

(d) means or controlling said computer system to carry out a determination and evaluation for each of said oligonucleotide sequences a value for at least one parameter that is predictive of the ability of each of said oligonucleotide sequences to hybridize to said target nucleotide sequence,

(e) means for storing said parameter values,

(f) means for controlling said computer system to carry out an identification, from said stored parameter values, a subset of oligonucleotide sequences within said number of non-identical oligonucleotide sequences based on an examination of said parameter,

(g) means for storing said subset of oligonucleotide sequences,

(h) means for controlling said computer system to carry out an identification of oligonucleotide sequences in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and for ranking said oligonucleotides based on the size of said clusters of oligonucleotides,

(i) means for storing said oligonucleotide sequences in said subset,

(j) means for controlling said computer system to select, for a cluster, a hybridization oligonucleotide wherein the hybridization of said hybridization oligonucleotide is predicted by the presence of said hybridization oligonucleotide in said cluster and

(k) means for outputting data relating to said oligonucleotide sequences in said subset.

147. (previously presented) A computer system according to claim 146 wherein the identified subset of oligonucleotide sequences is electronically transferred to an oligonucleotide array manufacturing system.

148. (previously presented) A method for selecting a hybridization oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying a predetermined number of non-identical oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being chosen to sample a length of said nucleotide sequence,

(b) determining and evaluating for each of said oligonucleotides at least one parameter that is predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence,

(c) selecting a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides based on an examination of said parameter,

(d) identifying oligonucleotides in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and ranking said clustered oligonucleotides based on the size of said clusters of oligonucleotides and selecting a subset of said clustered oligonucleotides, and

(e) selecting, for said subset of said clustered oligonucleotides, a hybridization oligonucleotide wherein the hybridization of said hybridization oligonucleotide is predicted by the presence of said hybridization oligonucleotide in said subset of said clustered oligonucleotides.

149. (previously presented) A method according to Claim 148 wherein the subset of said clustered oligonucleotides are selected to statistically sample the cluster.

150. (previously presented) A method according to Claim 148 wherein said non-identical oligonucleotides are of identical length N.

151. (previously presented) A method according to Claim 150 wherein said non-identical oligonucleotides are spaced one nucleotide apart, said predetermined number comprising L-N+1 oligonucleotides, where L is the length of the hybridizable sequence.

152. (previously presented) A method according to Claim 148 wherein said parameter is selected from the group consisting of composition factors, thermodynamic factors, chemosynthetic efficiencies and kinetic factors.

153. (previously presented) A method according to Claim 148 wherein said parameters are determined for said oligonucleotides by means of a computer program.

154. (previously presented) A method according to Claim 148 wherein said oligonucleotides are attached to a surface.

155. (previously presented) A method according to Claim 148 wherein said oligonucleotides are DNA or RNA or comprise chemically modified nucleotides.

156. (previously presented) A method according to Claim 148 wherein said target nucleotide sequence is RNA or DNA or comprise chemically modified nucleotides.

157. (previously presented) A method for selecting a hybridization oligonucleotide to hybridize to a target nucleotide sequence, said method comprising:

(a) identifying a predetermined number of non-identical oligonucleotides within a nucleotide sequence that is hybridizable with said target nucleotide sequence, said oligonucleotides being chosen to sample a length of said nucleotide sequence,

(b) determining and evaluating for each of said oligonucleotides at least one parameter that is predictive of the ability of each of said oligonucleotides to hybridize to said target nucleotide sequence, wherein said parameter is (1) a composition factor selected from the group

consisting of mole fraction (G+C), percent (G+C), sequence complexity, and sequence information content, (2) a chemosynthetic efficiency selected from the group consisting of coupling efficiencies and overall efficiency of the synthesis of a target nucleotide sequence or an oligonucleotide probe, (3) derived from a factor by mathematical transformation of said factor wherein said factor is predictive of the ability of an oligonucleotide to hybridize with a target nucleotide sequence, or (4) the difference between the predicted duplex melting temperature corrected for salt concentration and the temperature of hybridization of each of said oligonucleotides with said target nucleotide sequence,

(c) selecting a subset of oligonucleotides within said predetermined number of non-identical oligonucleotides based on an examination of said parameter,

(d) identifying oligonucleotides in said subset that are in clusters along a region of said nucleotide sequence that is hybridizable to said target nucleotide sequence and

(e) selecting, for a cluster, a hybridization oligonucleotide wherein the hybridization of said hybridization oligonucleotide is predicted by the presence of said hybridization oligonucleotide in said cluster.

158. (previously presented) A method according to Claim 157 which comprises ranking said oligonucleotides of step (d) based on the size of said clusters of oligonucleotides.

159. (previously presented) A method according to Claim 157 wherein said non-identical oligonucleotides are of identical length N.

160. (previously presented) A method according to Claim 157 wherein said non-identical oligonucleotides are spaced one nucleotide apart, said predetermined number comprising L-N+1 oligonucleotides, where L is the length of the hybridizable sequence.

161. (previously presented) A method according to Claim 157 which further comprises selecting a subset of said clustered oligonucleotides of step (d).

162. (previously presented) A method according to Claim 161 wherein said subset consists of any number of oligonucleotides within said cluster of oligonucleotides.

163. (previously presented) A method according to Claim 157 wherein said parameters are determined for said oligonucleotides by means of a computer program.

164. (previously presented) A method according to Claim 157 wherein said oligonucleotides are attached to a surface.

165. (previously presented) A method according to Claim 157 wherein said oligonucleotides are DNA or RNA or comprise chemically modified nucleotides.